Application No: 09/443,842
Filing Date: November 19, 1999
Group Art Unit: 1651
Examiner: V. Afremova
Atty Docket No: 22727-41

CLEAN COPY OF ALL CLAIMS

B1

37. (New) A method for preserving mammalian cells having lipid membranes for dry storage, comprising:

a. Reversibly porating the lipid membranes of the mammalian cells;

b. Loading the porated mammalian cells with a bio-preservation agent having bio-preservation properties to a predetermined intracellular concentration sufficient for preserving the cellular material, the predetermined intracellular concentration of the agent being less than or equal to about 1.0 M;

c. Preparing the bio-preservation agent loaded mammalian cells for storage by drying the cells without the use of a freezing step and wherein the drying is carried out at non-cryogenic temperatures; and

d. Dry storing the prepared mammalian cells so that hydration of the mammalian cells restores them to a viable state in which the mammalian cells survive and grow.

38. (New) The method of claim 37, wherein the lipid membranes are porated using a membrane toxin.

39. (New) The method of claim 38, wherein the lipid membranes are reversibly porated using a *Staphylococcus aureus* α-toxin.

40. (New) The method of claim 39, wherein the lipid membranes are reversibly porated using H5 α-toxin.

41. (New) The method of claim 40, wherein the step of reversibly porating the lipid membranes comprises forming pores of at least about 2.0 nanometers in the lipid membranes.

42. (New) The method of claim 37, wherein the bio-preservation agent comprises a non-permeating sugar having bio-preservation properties.

43. (New) The method of claim 42, wherein the sugar having bio-preservation properties is selected from a group consisting of trehalose, sucrose, glucose, and maltose.

44. (New) The method of claim 43, wherein the bio-preservation agent consists essentially of the sugar selected from the group consisting of trehalose, sucrose, glucose, and maltose.

45. (New) The method of claim 37, wherein the mammalian cells are loaded with an intracellular concentration of bio-preservation agent less than or equal to about 0.4 M.

46. (New) The method of claim 37, wherein the drying is accomplished by vacuum or air drying.

47. (New) The method of claim 43, wherein the bio-preservation agent further comprises a penetrating cryoprotective agent.

48. (New) The method of claim 47, wherein the bio-preservation agent comprises a penetrating cryoprotective agent selected from the group consisting of DMSO, glycerol and ethylene glycol.

49. (New) A method for cryopreserving mammalian cells having lipid membranes, comprising:

a. Reversibly porating the lipid membranes of the mammalian cells;

b. Loading the porated mammalian cells with an agent having bio-preservation properties to a predetermined intracellular concentration sufficient for preserving the cellular

material, the agent comprising a non-permeating preservation agent and the predetermined intracellular concentration of the agent being less than or equal to about 1.0 M;

c. Preparing the bio-preservation agent loaded mammalian cells for storage by freezing the cells to cryogenic temperatures; and

d. Cold storing the prepared mammalian cells so that thawing of the mammalian cells restores them to a viable state in which the mammalian cells survive and grow.

50. (New) The method of claim 49, wherein the lipid membranes are porated using a membrane toxin.

51. (New) The method of claim 50, wherein the lipid membranes are reversibly porated using a *Staphylococcus aureus* α-toxin.

52. (New) The method of claim 51, wherein the lipid membranes are reversibly porated using H5 α-toxin.

53. (New) The method of claim 52, wherein the step of reversibly porating the lipid membranes comprises forming pores of at least about 2.0 nanometers in the lipid membranes.

54. (New) The method of claim 49, wherein the bio-preservation agent comprises a non-permeating sugar having bio-preservation properties.

55. (New) The method of claim 54, wherein the sugar having bio-preservation properties is selected from a group consisting of trehalose, sucrose, glucose, and maltose.

56. (New) The method of claim 55, wherein the bio-preservation agent consists essentially of the sugar selected from the group consisting of trehalose, sucrose, glucose, and maltose.

57. (New) The method of claim 49, wherein the mammalian cells are loaded with an intracellular concentration of bio-preservation agent less than or equal to about 0.4 M.

58. (New) The method of claim 49, wherein the freezing is accomplished by exposing the bio-preservation agent loaded mammalian cells to an environment maintained at a cryogenic temperature.

59. (New) The method of claim 58, wherein the bio-preservation agent loaded mammalian cells are plunge frozen.

60. (New) The method of claim 55, wherein the bio-preservation agent further comprises a penetrating cryoprotective agent.

61. (New) The method of claim 60, wherein the bio-preservation agent comprises a penetrating cryoprotective agent selected from the group consisting of DMSO, glycerol and ethylene glycol.

62. (New) A method for preserving mammalian cells having lipid membranes, comprising:

a. Reversibly porating the lipid membranes of the mammalian cells;

b. Loading the porated mammalian cells with an agent having bio-preservation properties to a predetermined intracellular concentration sufficient for preserving the cellular material, the agent consisting essentially of a non-permeating sugar and the predetermined intracellular concentration of the agent being less than or equal to about 1.0 M;

c. Preparing the bio-preservation agent loaded mammalian cells for storage by a method selected from the group consisting of cryopreserving, freeze drying, and drying without the use of a freezing step; and

d. Storing the prepared mammalian cells so that they can be recovered to a viable state in which the mammalian cells survive and grow.

63. (New) The method of claim 62, wherein the lipid membranes are porated using a membrane toxin.

64. (New) The method of claim 63, wherein the lipid membranes are reversibly porated using a *Staphylococcus aureus* α-toxin.

65. (New) The method of claim 64, wherein the lipid membranes are reversibly porated using H5 α-toxin.

66. (New) The method of claim 65, wherein the step of reversibly porating the lipid membranes comprises forming pores of at least about 2.0 nanometers in the lipid membranes.

67. (New) The method of claim 62, wherein the sugar having bio-preservation properties is selected from a group consisting of trehalose, sucrose, glucose, and maltose.

68. (New) The method of claim 62, wherein the mammalian cells are loaded with an intracellular concentration of bio-preservation agent less than or equal to about 0.4 M.

69. (New) The method of claim 62, wherein the bio-preservation agent loaded mammalian cells are prepared for storage by freezing to cryogenic temperatures sufficient to permit cryogenic storage of the mammalian cells.

70. (New) The method of claim 62, wherein the bio-preservation agent loaded mammalian cells are prepared for storage by freeze drying to a level sufficient to permit dry storage of the mammalian cells.

71. (New) The method of claim 70, wherein the bio-preservation agent loaded mammalian cells are plunge frozen to a cryogenic temperature.

72. (New) The method of claim 62, wherein the bio-preservation agent loaded mammalian cells are prepared for storage by vacuum or air drying to a level sufficient to permit dry storage of the mammalian cells.

73. (New) A method for preserving nucleated cells having lipid membranes, comprising:

a. Reversibly porating the lipid membranes of the nucleated cells;

b. Loading the porated nucleated cells with an agent having bio-preservation properties to a predetermined intracellular concentration sufficient for preserving the cellular material, the predetermined intracellular concentration of the agent being less than or equal to about 1.0 M;

c. Preparing the bio-preservation agent loaded nucleated cells for storage by a method selected from the group consisting of cryopreserving, freeze drying, and drying without the use of a freezing step; and

d. Storing the prepared nucleated cells so that they can be recovered to a viable state in which the mammalian cells survive and grow.

74. (New) The method of claim 73, wherein the cellular material comprises nucleated mammalian cells.

75. (New) The method of claim 74, wherein the cellular material is selected from the group consisting of hepatocytes, fibroblasts, chondrocytes, keratinocytes, islets of Langerhans and hematopoeitic cells.

76. (New) The method of claim 73, wherein the lipid membranes are porated using a membrane toxin.

77. (New) The method of claim 76, wherein the lipid membranes are reversibly porated using a *Staphylococcus aureus* α-toxin.

78. (New) The method of claim 77, wherein the lipid membranes are reversibly porated using H5 α-toxin.

79. (New) The method of claim 78, wherein the step of reversibly porating the lipid membranes comprises forming pores of at least about 2.0 nanometers in the lipid membranes.

80. (New) The method of claim 73, wherein the bio-preservation agent comprises a non-permeating sugar having bio-preservation properties.

81. (New) The method of claim 80, wherein the sugar having bio-preservation properties is selected from a group consisting of trehalose, sucrose, glucose, and maltose.

82. (New) The method of claim 81, wherein the bio-preservation agent consists essentially of the sugar selected from the group consisting of trehalose, sucrose, glucose, and maltose.

83. (New) The method of claim 73, wherein the mammalian cells are loaded with an intracellular concentration of bio-preservation agent less than or equal to about 0.4 M.

84. (New) The method of claim 73, wherein the bio-preservation agent loaded mammalian cells are prepared for storage by freezing to cryogenic temperatures sufficient to permit cryogenic storage of the mammalian cells.

85. (New) The method of claim 73, wherein the bio-preservation agent loaded mammalian cells are prepared for storage by freeze drying to a level sufficient to permit dry storage of the mammalian cells.

86. (New) The method of claim 85, wherein the bio-preservation agent loaded mammalian cells are plunge frozen to a cryogenic temperature.

87. (New) The method of claim 73, wherein the bio-preservation agent loaded mammalian cells are prepared for storage by vacuum or air drying to a level sufficient to permit dry storage of the mammalian cells.

88. (New) The method of claim 81, wherein the bio-preservation agent further comprises a penetrating cryoprotective agent.

89. (New) The method of claim 88, wherein the bio-preservation agent comprises a penetrating cryoprotective agent selected from the group consisting of DMSO, glycerol and ethylene glycol.

90. A method for preserving mammalian cells having lipid membranes, comprising:

a. Applying a membrane toxin to reversibly porate the lipid membranes of the mammalian cells;

b. Loading the porated mammalian cells with an agent having bio-preservation properties to a predetermined intracellular concentration sufficient for preserving the cellular material, the agent comprising a non-permeating sugar and the predetermined intracellular concentration of the agent being less than or equal to about 1.0 M;

c. Preparing the bio-preservation agent loaded mammalian cells for storage by a method selected from the group consisting of cyropreserving, freeze drying, and drying without the use of a freezing step; and

d. Storing the prepared mammalian cells so that they can be recovered to a viable state in which the mammalian cells survive and grow.

91. (New) The method of claim 90, wherein the lipid membranes are reversibly porated using a *Staphylococcus aureus* α-toxin.

92. (New) The method of claim 91, wherein the lipid membranes are reversibly porated using H5 α-toxin.

93. (New) The method of claim 92, wherein the step of reversibly porating the lipid membranes comprises forming pores of at least about 2.0 nanometers in the lipid membranes.

94. (New) The method of claim 90, wherein the non-permeating sugar is selected from a group consisting of trehalose, sucrose, glucose, and maltose.

95 (New) The method of claim 94, wherein the bio-preservation agent consists essentially of the sugar selected from the group consisting of trehalose, sucrose, glucose, and maltose.

96. (New) The method of claim 90, wherein the mammalian cells are loaded with an intracellular concentration of bio-preservation agent less than or equal to about 0.4 M.

97. (New) The method of claim 90, wherein the bio-preservation agent loaded mammalian cells are prepared for storage by freezing to cryogenic temperatures sufficient to permit cryogenic storage of the mammalian cells.

98. (New) The method of claim 90, wherein the bio-preservation agent loaded mammalian cells are prepared for storage by freeze drying to a level sufficient to permit dry storage of the mammalian cells.

99. (New) The method of claim 98, wherein the bio-preservation agent loaded mammalian cells are plunge frozen to a cryogenic temperature.

100. (New) The method of claim 90, wherein the bio-preservation agent loaded mammalian cells are prepared for storage by vacuum or air drying to a level sufficient to permit dry storage of the mammalian cells.

101. (New) The method of claim 94, wherein the bio-preservation agent further comprises a penetrating cryoprotective agent.

102. (New) The method of claim 101, wherein the bio-preservation agent comprises a penetrating cryoprotective agent selected from the group consisting of DMSO, glycerol and ethylene glycol.